Original Article

Effect of Different Media, Photoperiod in Seeds Germination and Effects of Plant Growth Regulators on Seedling Growth of Endangered Medicinal Plant *Psoralea corylifolia* Linn.

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ABSTRACT

The study investigated the effects of light and different media on the germination of the seeds of endangered medicinal plant *Psoralea corylifolia* and its growth. The fastest and highest percentage of seed germination was achieved using MS medium as compared to B_5 medium and Whites medium. Germination of seeds of *Psoralea corylifolia* was enhanced under light conditions. 80% seed germination was observed 10^{th} day after sowing. Best results were obtained on MS medium containing BAP (15 µM) with NAA (20.0 µM) and Kn (15.0 µM) for sub sequent growth of seedlings within six weeks, generating shoots (6.5 shoots per explants).

Keywords: *Psoralea corylifolia*, explants, seed germination, plant growth regulator.

INTRODUCTION

The temperature and light are the most important environmental factors that promote the seed germination in the soil when water is available. The germination of the seeds is a complex process where several reactions and individual factors are involved, every process affected by the light[1]. The light affects the germination and the state of dormancy of the seeds and the seasonal changes of the dormancy state of the seeds of some species is directly related to the seasonal temperature changes[2]. Some species can present the seeds with the light requirement for the germination at one temperature and in another, the light insensitivity[3].

Psoralea corylifolia is an important medicinal plant species, used in the treatment of various skin diseases. Wild population of *Psoralea* has declined due to poor rate of seed germination[4,5]. A conventional method of propagation of

Psoralea corylifolia through seed is not an adequate solution to meet the demand of pharmaceutical industry. The seeds have a hard seed coat and therefore its germination is poor (5-7%), and viability of seeds is limited only to a few weeks, the seedling emerges from the germination of seeds, leads to death in natural environment. Due to short span viability *In vitro* seeds (green seeds) germination is one of the most beneficial process to save this elite vine. Tissue culture offers an alternative propagation method would be beneficial in accelerating large scale multiplication, improvement and conservation of *Psoralea corylifolia.*

Psoralea corylifolia grows as winter season weed. It is an erect annual herb, 30 - 180 cm. Leaves are broadly elliptic, arranged in racemes. Flowers are yellow or bluish purple colour and Seeds are smooth, adhering to the pericarp, dark brown and elongated[6]. *Psoralea corylifolia* contains coumarins, flavonoids, and merotepenes such as psoralen, isopsoralen, neo-bavaislfoavone, bovachin, bavaislfo-avone, bavachromene, psoralidin, coryli-folinin, bavachinin, bavachalcone[7].

The germination of *Psoralea corylifolia* seeds is highly erratic and staggered probably due to hard seed coat resulting in a low biomass and reproductive yield. The seeds of *Psoralea corylifolia* have staggered germination due to hard seed coat almost similar to some other sp. of the family. Seed required pretreatment of scarification to improve and enhance germination the untreated seeds shows very poor germination while treated give better result.

Thus it was considered in the present investigation, of interest to investigate the response of its seed to the varying levels of light and germination media. Various germination media are used for germinating to seeds. For good result the medium must be sufficiently firm and dense to hold the seeds in place during germination. It must retain enough moisture so that watering does not have to be too frequent. Hence the study on germination at different light and media was carried out to find out the best thermal regime and suitable germination media to maximize seed germination.

MATERIALS AND METHODS

Collection of the seeds

The seeds were collected in the month of November-December, 2011 from mature growing plant of *Psoralea corylifolia* inside Herbal garden, Hoshangabad (M.P.).

Surface sterilization

Seeds were thoroughly washed under running tap water for 30 min then treated with 5% tween-twenty for 5 minutes with constant stirring followed by 3-4 rinses in sterile distilled water and further treated with an antifungal agent (Bavistin) for 1 hours and were further with detergent for 10 min. and rinsed 4-5 times tap water. These all steps were carried out outside the laminar air flow chamber. Seeds were dipped into 0.1% (w/v) freshly prepared mercuric chloride solution for 8 minutes, and then washed with 4-5 times in sterile double distilled water.

Effect of different media on seed germination

Surface-sterilized seeds were inoculated aseptically on three basal media namely MS (Murashige and Skoog, 1962), B5 (Gamborgs *et al*., 1968) and WH (Whites, 1963). Seeds were also inoculated on the concentration of half strength MS media containing 3% sucrose and gelled with 0.8% agar having pH 5.7 in culture bottles by adding Hcl and NaoH prior to autoclaving at 15psi for 20 min. at 121°C. The cultures incubated at 24-25^{0}C under dark for two days and under fluorescent light with a 16/8 light/dark photoperiod[8].

Effect of light and dark on seeds germination

The explants (seeds) were inoculated on MS (full) medium and incubated light and dark period. Medium were supplemented with 3% w/v sucrose, adjust 5.5 to 5.8 pH (1N HCl or 1N NaOH) and solidified with 0.8 % agar than Autoclaved 121^0C for 15-20 min, poured into culture bottles. All cultures were incubated in both light (16 h photoperiod under cool white flouresent tubes at 55µmol$m^{-2}s^{-2}$) and dark conditions to access the effect of light on germination.

Effects of plant growth regulators on seedling growth

After 20 days old *in vitro* seedlings were used as a source of explants were cultured on MS basal medium supplemented with different concentrations and combinations of plant growth regulators, 6-Benzylaminopurine (BAP: 1.0 -20.0 µM), Kinetin (Kn: 2.5- 18 µM), 1-Naphthaleneacetic acid (NAA: 3.0- 20.0 µM) and B5 vitamins + 2mg / ltr. Glycine (MBG) formulations for shoot proliferation and multiplication. All cultures were incubated under 16 h photo periods with light intensity of 55µmol$m^{-2}s^{-2}$ provided by cool white fluorescent lamps (Phillips, India) at 25 ± 2°C. All the cultures were transferred to fresh medium after 2-3 week duration.

RESULT AND DISCUSSION

After 8-10 days seeds were started germinating (Fig 1.1). The fastest and highest percentage of seed germination was achieved using MS medium. Seeds on MS medium germinated in 2–3 weeks **(**Fig 1.2, 1.3) compared with 5–6 weeks on B5 medium. Seedling development was also superior on MS medium. The highest percentage of seed germination (95%) were recorded in the MS full basal media followed by MS half basal media were (86%). Only 35% seed were germinated on Whites medium (Table 1). After 20 days *in vitro* seedlings were used as a source of explants for further experiments that is on callus development and *in vitro* micropropagation.

The importance of NH_4^+ and NO_3^- ions (individually or in combination) during *in vitro* germination of seeds as a source of nitrogen is well established[9,10]. The source of nitrogen in MS medium is ammonium nitrate, whereas in WH and B_5 it is in the form of ammonium sulphate. Furthermore, NO_3^- in the form of calcium nitrate ($CaNO_3$) is present in WH and B_5 media. Alan reported that $CaNO_3$ might lower nitrogen content compared with other mineral elements[11]. The presence of ammonium nitrate in MS medium may explain the high germination rate because NH_4^+ is readily assimilated during the initial stages of development and greatly influences growth and differentiation[12,13]. The high germination and strong further development in MS medium could be attributed to the fact that MS medium is also especially rich in both macro- and micro-nutrients.

Seeds germinated poorly especially in the dark with a maximum of 40% after 10 days (Table 2). Germination of seeds of *Psoralea corylifolia* was enhanced under light conditions. 80% seed germination was observed 10th day after sowing. Similar result is also found in *O. gratissimum*[14]. It has long been established that light sensitivity of seeds operates through the phytochrome pigment systems[15,16]. The dormancy challenge on the germination of *Psoralea corylifolia* appears to be a combination of hard seed coat, the presence of germination inhibitors and a light requirement. Hence the release of dormancy from these seeds can only be achieved when the restriction to the entry of water and oxygen is eliminated and that the inhibiting substances are prevented from affecting the biochemistry of germination in these seeds. This would consequently trigger other processes that lead to embryo germination.

Germinated seeds were isolated and transferred into different growth hormones with MS salt under photoperiod 16/8 hour's light/dark. Maximum shoot induction from cotyledonary node was observed from 18-d-old seedling explant compared to 6-12, and 24-d-old explants. Explant age plays a significant role to induce multiple shoots in a number of plants, including *Cercis Canadensis*[17,18]. Best results were obtained on MS medium containing BAP (15 µM) with NAA (20.0 µM) and Kn (15.0 µM) for sub sequent growth of seedlings within six weeks, generating shoots (6.5 shoots per explant). Seedling growth and developed 2 to 6 shoots at all concentration of BAP, Kn and NAA (alone and in combination) in (Table 3). MS medium containing BAP was more effective than kinetin and NAA for seedling growth as experiment. Increasing concentrations of Kn (>20 µM) and NAA (>25 µM) decreased shoot number and length. Decreasing the concentration of Kn (<10 µM) and NAA (<10 µM) also decreased shoot number. Reddy *et al*, in 1998 reported that kinetin did not improve significantly the shoot length and the number of proliferating shoots[19]. Superiority of BAP and kinetin in combination has been found for micro propagation of other woody perennials reported by Das *et al*. (1996); Komalavalli and Rao, in (1997)[20,21].

CONCLUSION

From the above findings, it may be concluded that MS medium was best for seed germination in comparison to that of other media and germination of seeds of *Psoralea corylifolia* was enhanced under light conditions. Hence media, growth regulators and light plays a very important role for rapid propagation of *Psoralea corylifolia* a medicinally important endangered plant.

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Table 1. Different Media used for seed Germination of *Psoralea corylifolia*

Medium	% of seed germination	Mean Length of seedling (cm)
MS (full strength)	95	2.1 ± 0.08
MS (half strength)	86	1.5 ± 0.02
B5 (full strength)	45	0.54 ± 0.01
B5 (half strength)	40	0.48 ± 0.04
WH	35	0.69 ± 0.03

Table 2. Percentage germination of *Psoralea corylifolia* seeds after 10 days incubation under light and dark

Period (days)	Percentage of germination	
	Light	Dark
2	0.0	0.0
4	20.0	0.0
6	40.0	0.0
8	70.0	30.0
10	80.0	40.0

Table 3. Effect of plant growth regulators on seedling growth from germinated seed after 6 weeks

S. No	Media Code	MS + Auxins / cytokinin (µ*M*)			Shoot regeneration (%)	No. of shoots
		BAP	Kn	NAA		
1	SMS 1	1.0	-	-	20	1.95 ± 1
2	SMS 2	3.0	-	-	35	2.1± 2
3	SMS 3	6.0	-	-	70	2.92 ± 2
4	SMS 4	9.0	-	-	75	3.01± 2
5	SMS 5	12.0	-	-	75	4.95± 2
6	SMS 6	15.0			90	5.12 ± 3
7	SMS 7	18.0	-	-	40	2.92 ± 2
8	SMS 8		2.3	-	45	1.33 ± 2
9	SMS 9		5.0	-	50	2.2 ± 4
10	SMS10		10.0	-	65	3.1 ± 1
11	SMS 11		15.0	-	80	4.1 ± 3
12	SMS 12		20.0	-	50	3.5 ± 2
13	SMS 13	-		3.0	30	1.43 ± 1
14	SMS 14	-		5.0	44	2.6 ± 3
15	SMS 15	-		10.0	65	3.10 ± 2
16	SMS 16	-		15.0	82	4.3 ± 3
17	SMS 17	-		20.0	85	3.1 ± 1
18	SMS 18			25.0	60	3.0± 1
19	SMS 19	6.0	10.0	-	30	2.01± 2
20	SMS 20	9.0	10.0	-	44	2.1± 2
21	SMS 21	12.0	10.0	-	65	3.2 ± 3
22	SMS 22	15.0	10.0	-	82	4.12 ± 2
23	SMS 23	6.0	15.0	-	60	3.5 ± 2
24	SMS 24	9.0	15.0	-	80	6.1 ± 2
25	SMS 25	12.0	15.0	-	86	6.9± 2
26	SMS 26	15.0	15.0	-	90	7.4± 2
27	SMS 27	6.0	15.0	15.0	80	3.6± 2
28	SMS 28	9.0	15.0	15.0	83	4.9 ± 1
29	SMS 29	12.0	15.0	15.0	85	5.1± 2
30	SMS 30	15.0	15.0	15.0	85	5.5 ± 2
31	SMS 31	15.0	15.0	20.0	95	6.5 ± 2

 ISSN 2321 – 2748

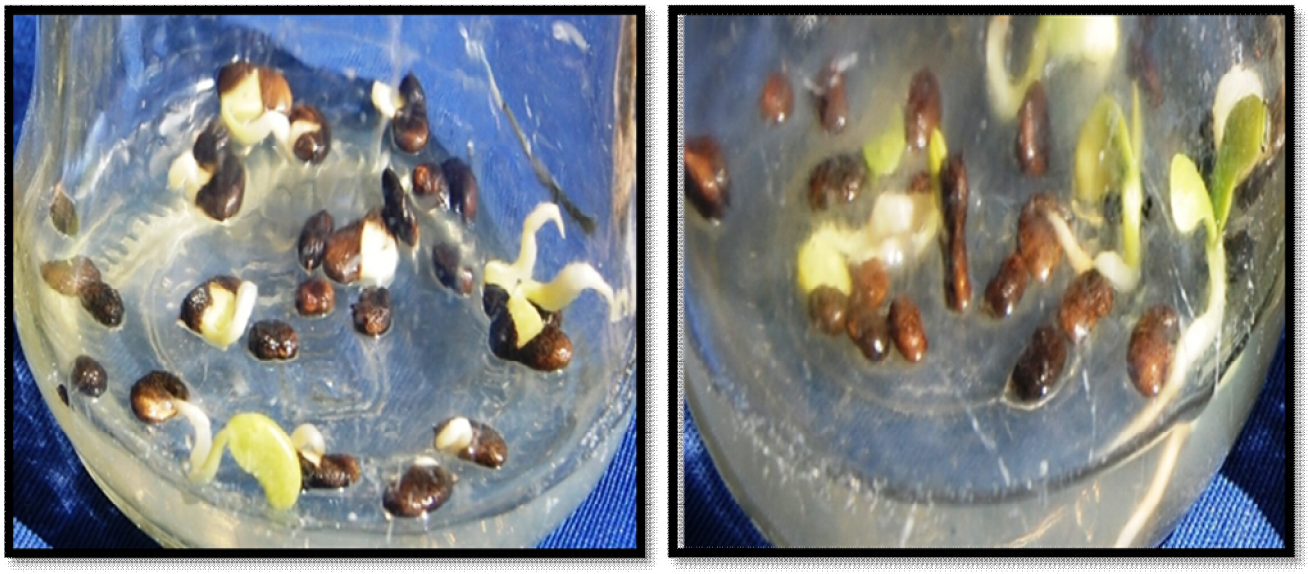

Figure 1.1. Seedlings of 8-10 days of *Psoralea corylifolia*

Figure 1.2. Seedlings of 2–3 weeks of MS media of *Psoralea corylifolia.*

 ISSN 2321 – 2748

Figure 1.3. Seedlings of 4–6 weeks of MS media of *Psoralea corylifolia*